AMENDMENT TO SPECIFICATION

Please amend the following paragraphs of the specification as follows:

[0006] International patent application PCT/EP00/08537 describes such an exception. Synthesis and export of the hirudin derivative lepirudin, the active ingredient of the pharmaceutical ~~Refludan~~REFLUDAN™, by *E. coli* in gram quantities was successful when using specific signal sequences for exporting. German Application No. 100 33 195.2 (unpublished) describes a bifunctional protein composed of hirudin and hirudin derivatives and of factor Xa inhibitor from ticks and derivatives thereof. Said protein can likewise be synthesized and exported by E. coli with high yields. In addition, it was then surprisingly found that hirudin is exported with high yields not only as a fusion protein with TAP but also as part of a fusion protein with polypeptides such as proinsulin derivatives, that it is biologically active and that surprisingly a fusion partner such as proinsulin is present in the correct three-dimensional structure. This unexpected result leads to the possibility of more cost-effective production of, for example, insulin by bacterial host/vector systems, since the step of in vitro refolding after intracellular expression, which is associated with losses in yield which are not negligible, can be dispensed with and in this way a simpler protein purification process results. Another advantage is that chaotropic aids added to dissolve the fusion protein in traditional processes for the production of insulin in E. coli are not required. Ecologically, this leads to less environmental pollution by avoiding the corresponding waste.

[0008] The invention includes the use of hirudin and hirudin variants for the formation of fusion proteins, for example with simian proinsulin or derivatives thereof. Particular aspects of the invention use one of the natural hirudin isoforms (the natural isoforms together are denoted "hirudin"). Natural isoforms are, for example, Val-Val-hirudin or Ile-Thr-hirudin. Other aspects of the invention use a variant of a natural hirudin isoform. A hirudin variant is derived from a natural hirudin isoform but contains, for example, additional amino acids and/or amino acid deletions and/or amino acid exchanges compared with the natural isoform. A hirudin variant may contain alternating peptide

segments of natural hirudin isoforms and new amino acids. Hirudin variants are known and are described, for example, in DE 3 430 556. Hirudin variants are commercially available in the form of proteins (~~Calbiochem~~CALBIOCHEM™ Biochemicals, Cat. No. 377-853, -950-960). The hirudin variant sequences are at least 40% homologous to lepirudin, such that 40% of the total amount of the 65 amino acids known from lepirudin should be found within the variant. The hirudin variant sequences may be even more homologous, such as at least about 60%, or at least about 80%, homologous to hirudin. The % homology is calculated by the Compare Program which is available from the ~~Wisconsin Package~~WISCONSIN PACKAGE™ distributed by the Genetics Computer Group; 575 Science Drive; Madison, Wis.

[0010] Insulin derivatives are derivatives of naturally occurring insulins, namely human insulin or animal insulins, which differ from the corresponding, otherwise identical naturally occurring insulin by substitution of at least one naturally occurring amino acid residue and/or addition of at least one amino acid residue and/or organic residue. It is understood that the term insulin defines a polypeptide composed out of a B- and A-chain. The insulin derivative may be at least 60% homologous to a naturally occurring insulin. The insulin derivative may be even more homologous, such as at least about 75%, or at least about 90%, homologous to a naturally occurring insulin. The % homology is calculated by the Compare Program, which is available from the ~~Wisconsin Package~~WISCONSIN PACKAGE™ distributed by the Genetics Computer Group; 575 Science Drive; Madison, Wis.

[0079] Example 2 of the patent application PCT/EP00/08537, which is incorporated by reference herein in its entirety, describes an expression vector which allows expression and secretion of ~~Refludan~~REFLUDAN™, into the medium used for E. coli via the signal sequence of the Pseudomonas fluorescens oprF gene product (De, E. et al., FEMS Microbiol Lett.127, 263 -272, 1995, which is incorporated by reference herein in its entirety. This vector served to construct a ~~Refludan~~REFLUDAN™-GNSAR-simian proinsulin fusion protein (GNSAR=SEQ ID NO.: 1) and was denoted pBpfu_hir.

[0087] Two standard polymerase chain reactions were carried out using the Hir_insf1/Insu11HindIII primer pair with plasmid pINT90d as template and the pfuf1/Hir_insrev primer pair with plasmid pBpfu_hir as template. To perform the reactions the ~~Advantage~~ADVANTAGE™-HF PCR Kit (Clontech Cat#K1909-1) was used. The reaction volume was 50 µl containing 1 µl polymerase, 5-10 ng template and about 100 ng of primer. 25 cycles: 30" at 95° C., 30" at 52° C. and 30" at 72° C. were run. The products of both reactions were isolated and about 5% of the yields were combined and converted in a third polymerase chain reaction which was run under the same conditions with primers pfuf1/Insu11HindIII. The result was a DNA product which contained the sequence signal (partially)-lepirudin-GNSAR-simian proinsulin. The DNA fragment was converted using restriction enzymes BamHI and HindIII (according to the manufacturer's protocol), with BamHI cleaving in the lepirudin sequence and HindIII at the 3' end of the proinsulin-encoding sequence.

[0100] The peptide sequence was translated into DNA by the GCG program Backtranslate (~~Wisconsin Package~~WISCONSIN PACKAGE™ Version 10.1, Genetics Computer Group (GCG), Madison, Wis.) using the E. coli high codon usage criteria.

[0111] The hirudin concentration of the supernatant of Example 5 was determined according to the method of Grießbach et al. (Thrombosis Research 37, pp. 347-350 1985, which is incorporated by reference herein in its entirety). For this purpose, ~~Refludan~~REFLUDAN™ standard was included in the measurements in order to establish a calibration curve from which the yield in mg/l was determined directly. The biological activity (unfolded molecules are not active) was also a direct measure for correct folding of the proinsulin component of the fusion protein. Alternatively, although not conducted as part of this Example, it is possible to use a proteolytic Staphylococcus aureus digestion and subsequent analysis in an RP-HPLC system in order to perform a peptide mapping to determine the correct S-S bridge formation.

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